megakaryocytes. A weaker but undoubtedly positive reaction was observed in the cytoplasm of neutrophilic and eosinophilic myelocytes, plasmacytes and reticular cells. In the human material, cells which could be recognized as proerythroblasts and basophilic erythroblasts presented a juxtanuclear positively reacting zone. More mature forms did not stain.

c) *Thymus and lymphatic glands.* A positive reaction was observed only in the plasmacytes and reticular cells. Lymphocytes were always negative in these organs. In the plasmacytes the reaction was localized in a juxtanuclear zone, diffusely or in granules. Granulocytes when present reacted positively.

d) *Spleen.* A red stain denoting a positive reaction could be observed in the megakaryocytes, neutrophilic and eosinophilic granulocytes, macrophages, plasmacytes and reticular cells. These cells stained similarly as the above described ones.

Discussion. The positive plasmal reaction observed indicates the presence of plasmalogens (glyceryl-phosphoryl-ethanolamine and possibly analogous compounds (Lovern)(2) in the blood and hemopoietic organ cells. The positivity of the reaction in the cytoplasm of the megakaryocytes and in the platelets, adds more cytochemical evidence to the megakaryocytic origin of the platelets. The physiological significance of the presence of these compounds in the cells is unknown, but perhaps it might be connected with a protective mechanism(3). This reaction has been applied to pathological human material and a detailed account of the results obtained will be published elsewhere.

Summary. A positive cytoplasmic reaction was obtained by a modified technic of plasmal reaction in the following cells from human and rat blood and hemopoietic organs: megakaryocytes, platelets, neutrophils, eosinophils, lymphocytes, monocytes, plasmacytes, early erythroblasts and reticular cells.

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Effect of Protamine Sulfate in Neutralizing Coagulation Defect Produced by Paritol.* (19870)

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In the constant search for an ideal anticoagulant, many new compounds with potent anticoagulant ability have been tried clinically. One of these new compounds is paritol, a polysulfuric acid ester of polyanhydromannuronic acid. Its mode of action has not been definitely determined but it is thought to be similar to that of heparin. Animal experiments(5) have suggested the presence of an antithrombin inhibitor in the peripheral blood as the natural inactivator of paritol.

The use of powerful anticoagulants always carries the possibility of hemorrhage. In view of this it is desirable to have an adequate neutralizing agent which will assure some degree of safety from hemorrhage. Protamine sulfate has been used as an antagonist to the coagulation defect produced by heparin. It seemed advisable to determine the extent of this neutralizing effect on the coagulation defect produced by paritol. Protamine sulfate was found(4) to have a clear-cut neutralizing effect upon paritol activity in dogs and rab-

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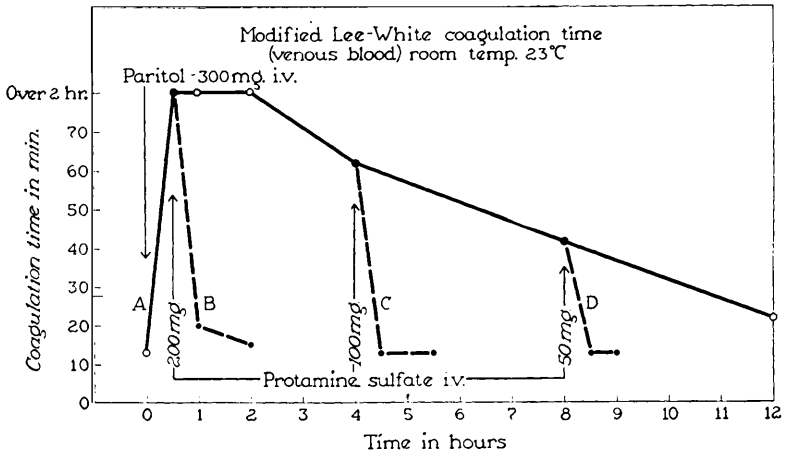

FIG. 1.

bits. The coagulation time of the blood in these animals returned to normal within 5 minutes after administration of protamine sulfate. If these results are directly applicable to clinical studies, then there would seem to be little risk of serious hemorrhage from such a powerful anticoagulant as paritol. There appears to be no report at this time on the neutralizing effect of protamine sulfate on the coagulation defect produced by paritol in human subjects.

Method of study. All subjects, 15 male and one female, had been hospitalized because of various types of vascular disease. The ages ranged from 21 to 82 years. The subjects presented no evidence of hepatic, renal, or hemorrhagic disease. In agreement with Sorenson and Wright(8) it was found that 300 mg of paritol injected intravenously produced and maintained a prolongation of the coagulation time of whole venous blood greater than 20 minutes for approximately 8 to 14 hours. Unless otherwise stated paritol† was injected intravenously in a dose of 300 mg throughout this investigation. Protamine sulfate was administered intravenously in a 1% solution. Immediately before the injection of paritol and for various intervals up to 24 hours after its administration the coagulation time was measured. When the coagulation time had returned to normal the subject was again given paritol. During this second period an attempt was made to study the effect of protamine sulfate in neutralizing the coagulation defect caused by paritol. After careful venipunctures had been performed the coagulation times were determined by a modified Lee-White method and by the Barker coagulochronometer(1). Since both methods produced similar results, the following discussion will refer to the modified Lee-White method. The average clotting time of the 16 subjects by the Lee-White technic was 12.5 minutes with a range of 11 to 16 minutes.

Results. The anticoagulant activity of paritol was rapid as evidenced by the coagulation defect produced within 30 minutes after its injection, and prolonged as noted by the 8 to 12 hours required for the coagulation time to return to normal (Fig. 1, curve *A*). An attempt was made to neutralize this coagulation defect when it was maximal and at various other intervals during its period of activity.

Fig. 1, curve *B*, illustrates the neutralizing effect of 200 mg of protamine sulfate administered 30 minutes after the injection of paritol. This is in striking contrast to the uninhibited anticoagulant effect of paritol as shown in curve *A*. Previous attempts with smaller amounts of protamine sulfate (100 to 150 mg) were not effective.

Fig. 1, curve *C*, is an example of the neutralizing effect of 100 mg of protamine sulfate upon the coagulation defect 4 hours after the administration of paritol. Smaller amounts

† Lot 432B supplied by courtesy of Wyeth, Inc., Philadelphia.

of protamine sulfate were found to be ineffective. Fig. 1, curve *D*, is an illustration of the response to 50 mg of protamine sulfate given 8 hours after the administration of paritol.

Comment. Protamine sulfate was found to exert a strong neutralizing effect on paritol activity. There were two substantial differences between its neutralizing effect upon paritol and upon heparin. Parkin and Kvale (3) found that the reaction between heparin and protamine sulfate was completed within 5 minutes. In the case of paritol it required a longer time. The other important difference was the large amount of protamine sulfate required to produce an adequate neutralizing effect upon the activity of paritol (Fig. 1, curves *B* and *C*). The use of such doses may possibly be hazardous because of the well-known toxic effects of protamine sulfate itself (2,6,7).

Two reactions occurred in our studies. Each began within 30 minutes after the injection of protamine sulfate during an attempt to neutralize the anticoagulant activity of paritol which had been given previously. They consisted of chills, fever, nausea, vomiting, burning epigastric pain and aching in the lumbar region of the back. These symptoms began to subside in a few hours and had disappeared completely within 4 days. The one unusual feature that was noted in these 2 cases was that the protamine sulfate had been administered in a more rapid manner than that recommended by other authors. This reaction did not occur when the protamine had been given 4 or 8 hours after the paritol had been given.

Summary. This study indicates that protamine sulfate is an active antagonist of paritol in the human subject. Larger doses than those used in neutralizing the effect of heparin are required to counteract the defect produced by paritol. Large amounts of protamine sulfate may produce toxic symptoms and therefore should be used with caution.

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A Colorimetric Method for Evaluating Chymotrypsin Inhibitors in Human Serum. (19871)

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Elevation of chymotrypsin inhibitor levels occurs in human blood serum with various pathological states(1), with pregnancy(2), and with certain types of mental disorders (3). Determinations of these inhibitor levels by the method of West and Hilliard(4), using homogenized milk as a substrate, can be used to follow the course of diseases such as cancer, and serve as a guide in evaluating various types of therapy(5-7).

Since the determination of chymotrypsin inhibitor levels by the method of West and Hilliard employs a nonsynthetic substrate (milk) and involves subjective end point determinations, it was decided to look for a simple synthetic substrate whose hydrolysis